February 17, 1954

Professor Adrien Albert
Australian National University
London, England

Dear Professor Albert:

First, let me belatedly acknowledge, with thanks, yourshipment of 5-amino acridine and its 1,2,3,4-tetrahydro derivative. I am sorry to have to report that both vials arrived badly smashed, but Dr. Bernstein was fortunately able to recover about 50 mg. of each compound from the ruins, and this was sufficient for the immediate purpose.

The studies that Dr. Aleck Bernstein (here on leave from the P.H.L.S. from London) has been pursuing, and for which we requested your favor of these two compounds, have reached a stage that might, I hope, engage your interest and for which we are, at any rate, in need of some counsel. As they bear directly on the biochemical specificity of the acridines, I could not think of any more competent authority than yourself.

Our interest in the problem stems from a study of the genetic determination of flagellar phase variation in Salmonella spp., viz. the alternation of serological type of H antigen shown by most (i.e. diphasic) strains. As some of our studies now indicate (reprints under separate cover), it appears that the numerous distinct phase-1 (specific phase) antigens are governed by alleles at one locus, comparable, in a sense, to the [illegible] alternatives in the human blood groups, while the phase-2 antigens are homologues referable to a second locus,-- somewhat on the order of the MBON alternatives. If I may stretch the analogy further, Salmonella behaves as if, in the human system, a given erythrocyte could, at any time, display either its Rh character, or its ABO character but not both. Our genetic interest comes from the fact that the descendants of a cell in a given phase are almost always in the same phase. At any rate, there was little else in the literature to suggest that the phase-1 and phase-2 antigens did in fact belong to distinct homology groups, except for the quasi-genetic argument of the usual occurrence of one alternative from each group in any given serotype of Salmonella.

In an obscure report, however, Sertic & Boulgakov (1936, C.R.Soc. Biol. 123:951-2) indicated that acriflavine would agglutinate Salmonella cultures in phase-2, but not in phase-1. It is difficult for me to understand why this work has been overlooked so completely, but we have seen no subsequent reference of any kind. At any rate, it proved to be quite easy to confirm this account and to include various controls not emphasized by S.&B. A considerable variety of types have been carefully examined, and in each case a phase-2 culture was agglutinated; phase-1 was not. S&B's technique is not entirely satisfactory: a preferred procedure is to set up the cultures as if for a tube agglutination with anti-H serum. The cells should be either live, or killed with Roccal (v.i.). The acriflavine is effective to a dilution of about 1:5000 to 1:1000. Salt is not required. The agglutination is specific over a range from pH 6-8. At 9 and above, both phases are agglutinated; at lower pH, the dye tends to crystallize but the agglutination may also be directly reversed. The reaction requires 2-4 hours at 37°, and closely resembles a serum H agglutination. Similarly, it

is negated by prior heating of the bacterial suspension. Non-flagellated variants are not agglutinated. A distinct type of agglutination may also be observed, especially with rough cultures, but like the corresponding "O" agglutination, it is granular rather than flocculent, stable to heat, and requires considerably longer incubation. To prove more definitely that the reaction directly involves the flagella, preparations of isolated flagella have been made (after Gard & Weibull)-- it looks as if these behave in the same sense as the bacteria from which they are separated.

We had hoped to investigate the reaction by following the binding of the dye, but there is (as you would have anticipated) an overwhelming uptake of acriflavine even by non-flagellated variants that would obscure the effects we might be interested in.

Of several antiseptics tested, Roccal proved most effective while preserving the specific reaction. Formaldehyde appears to react directly with the dye, but washed formalized cells reacted in either phase (presumably in consequence of the removal of free amino groups). Other antiseptics either abolished the reaction or interfered with its observation.

We may now come to the problem of chemical specificity. The previous results suggested the possibility that phase-2 flagella might simply be more acidic than phase-1, a point we hope to check by a simple cataphoretic test, However, while (purified) euflavine, proflavine, and (one sample of) acridine orange and 5-amino acridine were each specifically effective under our conditions, such bases as 1,2,3,4-tetrahydro-5-amino-acridine, p-rosaniline, and streptomycin were not! Protamine sulfate gave a nonspecific agglutination, as might be expected. (No interference with acriflavine on the part of p-rosaniline was observed!) For other purposes we had hoped to find a specific reagent that would not impair the viability of the bacteria, but as you will recognize, the effective compounds are all potent antiseptics at the concentrations used. (No differential bactericide of the two phases was found).

This is about the present status: there is the problem what would be worthwhile to do next. Our primary interest is not so much the specificity of the acridines as a demonstration of a chemical differential of the genetically distinct phases, but it is difficult to isolate the two problems. I had hoped, however, that you might find sufficient interest in the parallelism of the specificity here and in the bacteriostatic tests to offer some counsel. If it should be possible for you to suggest (and furnish) a limited number of additional compounds that might be non-toxic reagents, or offer decisive clues as to the basis of the specificity, we would be indebted to you for the opportunity to test them. However, if a more extensive survey is indicated, as may well be the case, it might be more appropriate and more convenient for it to be conducted under your personal supervision, if the prospect accords with your present plans and facilities. Let me emphasize the technical facility of the tests; needless to say, we will be delighted to furnish cultures and whatever else would be applicable.

Aside from the possibility of a limited survey of additional compounds, our plans with regard to the chemical aspects of the problem are limited to the completion of observations on isolated flagella and to some comparisons in an electrophoresis apparatus.

Professor Rubbo sends his regards; he will be familiar with our results at close hand and will be ready to consult with you about them if this should seem appropriate (when you have both returned to Australia). I mention this for fear of having inadvertently left out some important detail. Needless to say, any comment you may have will be most welcome.

Yours sincerely

Joshua Lederberg

2/20/54 P.S.

We have just learned, from inhibition experiments, that the flagella of either phase are equally competent to bind acriflavine, so that the problem now devolves on the far more elusive point of the physical state of the flagellar material that results in massive aggregation only of phase 2 ! We had hoped to uncover some clues from the extensive literature on the use of acriflavine for the diagnosis of smooth/rough cultures, but although this test is very widely applied, we have found nothing bearing on its mechanism; your book makes no reference to it, but can you acquaint us with any literature?

If I had not mentioned it before, I would be greatly indebted to you for an exchange of publications. A packet of some of my own papers is mailed under separate cover. To avert duplication, may I mention that Professor Rubbo has kindly been able to furnish I-IV of the "antibacterial activity" series and I of "toxicity", but not any of the others or more recent works.

On the basis of the arguments in your book "Selective Toxicity" (which I found to be beautifully written as well as informative), a few choices of additional compounds suggest themselves. Would you agree on:

1) 4-aminoquinoline. 2) its 2-styryl-derivative. (to verify the shape requirement)
and

3). 2-aminoacridine-3-carboxylic ac. or 4) 5-amino-2-hydroxyacridine
(to verify the role of pK --not yet tested, in fact, in any way in our material.) ?

But there is an insistent temptation to examine "just one more compound".

Yours sincerely,

J.L.